ELEGEN

The Next-Gen DNA Buyer's Guide:

From Design to Results Faster and More Reliably with Cell-Free Gene Synthesis

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Introduction

The age of next-generation DNA manufacturing is here. With enzymatic and chemical-based DNA synthesis technologies and innovative cell-free solutions for cloning and amplification, today's gene synthesis providers offer different capabilities and services to meet the growing demand for synthetic DNA products.

HOW DO YOU SELECT THE RIGHT VENDOR FOR YOUR NEXT PROJECT?

Every project's complexity, deadlines, and budget vary, though securing a reliable and rapid source of DNA is a top priority. In this guide, we'll help answer this question by covering a brief history of gene synthesis, where the market is today, and why the recent commercialization of cell-free gene synthesis, providing long, complex, NGS-verified DNA with speed to eliminate cloning, represents a critical leap forward in DNA manufacturing.

Specifically, we'll examine:

- **Conventional vs. cell-free approaches to gene synthesis**
- **The impact of cell-free gene synthesis in specific research applications**
- **Important considerations for your next synthetic DNA purchase**
- **Key differences between the top gene synthesis providers**
- **Tips for formulating your DNA buying strategy**

02
A Brief History of Gene Synthesis

More than 150 years after its discovery, DNA has become an icon as one of the most important discoveries in biological research. The journey from the discovery of DNA to the development of gene synthesis technologies is one of the most transformative in biology.

Despite successful synthesis of the first synthetic bacterial genome, the emergence of next generation sequencing and the completion of the Human Genome Project shifted the focus of innovation in the early 2000s from writing DNA to reading and editing it. As sequencing by synthesis was followed by nanopore sequencing and single-cell sequencing technologies, the use of zinc-finger nucleases (ZFNs) was followed by transcription activator-like effector nucleases (TALENs) and the CRISPR-Cas system.

Through the 2010s, gene synthesis only witnessed incremental changes including the miniaturization of phosphoramidite DNA synthesis on a silicon chip and the emergence of enzymatic DNA synthesis. While both advancements improve the efficiency and quality of single-strand DNA synthesis, researchers continued to rely on decades-old technologies to assemble and clone double-stranded DNA.

REIGNITING DNA INNOVATION

In the past few years, cell-free DNA cloning and amplification technologies have had a transformative impact on the field of gene synthesis by offering new methods for producing DNA outside of living organisms. Emerging gene synthesis providers have leveraged these technologies to address long-standing limitations of traditional cell-based cloning.

Here are some of the key benefits of cell-free gene synthesis:

- **Increased speed and efficiency**
- **Improved reliability and scalability**
- **Greater accuracy**

Todays next-gen gene synthesis providers are using advanced technologies to help organizations overcome the limitations and compromises of conventional gene synthesis. Combining novel enzymatic chemistries with cell-free cloning and amplification, researchers now have access to high-accuracy, high-complexity, multi-gene constructs in a matter of days.

The DNA Innovation Timeline

Early Discovery[1]

1869

DNA first discovered by Friedrich Miescher

1944

DNA demonstrated to be a hereditary material

1952-53

The first photo of hydrated DNA by Rosalind Franklin inspires the first model of DNA by Watson & Crick

Synthesis Exploration[2]

1960s – 1970s

The development of solid-phase peptide synthesis and oligonucleotide synthesis enables approaches to DNA synthesis

1972

DNA from 2 different organisms is spliced together for the first time, paving the way for genetic modification

1980s

Advancements in DNA synthesis, copying, and assembly with phosphoramidite chemistry, polymerase chain reaction (PCR), and polymerase chain assembly (PCA)

- **1983** PCR technique created

1990s

Enhanced oligo assembly methods enable the synthesis of longer genes

- **1996** Dolly the sheep is cloned from a non-embryonic cell
- **1996-1999** Preclinical development of adeno-associated virus type 2 (AAV2) as a gene therapy vector[3]

2000s

New hybrid and enzymatic approaches to DNA synthesis emerge

- **2003** The first synthesis of the entire viral genome is achieved
- **2004** Microchip- and microfluidics-based methods for DNA synthesis are developed
- **2008** The first synthesis of entire bacterial genome is achieved

Breakthroughs, Advancements, and Therapy Approvals

2010s

Advancements in cloning techniques using closed-end DNA, microarrays, Gibson Assembly, and gene synthesis

- **2011** Successful AAV8 gene therapy demonstrated for hemophilia B
- **2011-2014** Clinical development of CAR-T cell therapies for CD19+ lymphoid malignancies
- **2012-2013** CRISPR-Cas9 system developed to perform targeted gene editing
- **2017** FDA approves CD19-directed CAR-T cell therapy (Kymriah, Novartis) for the treatment of relapsed, refractory acute lymphoblastic leukemia
- **2018** FDA approves the first-ever RNA interference (RNAi) therapeutic to treat polyneuropathy (Onpattro, Alnylam Pharma.)

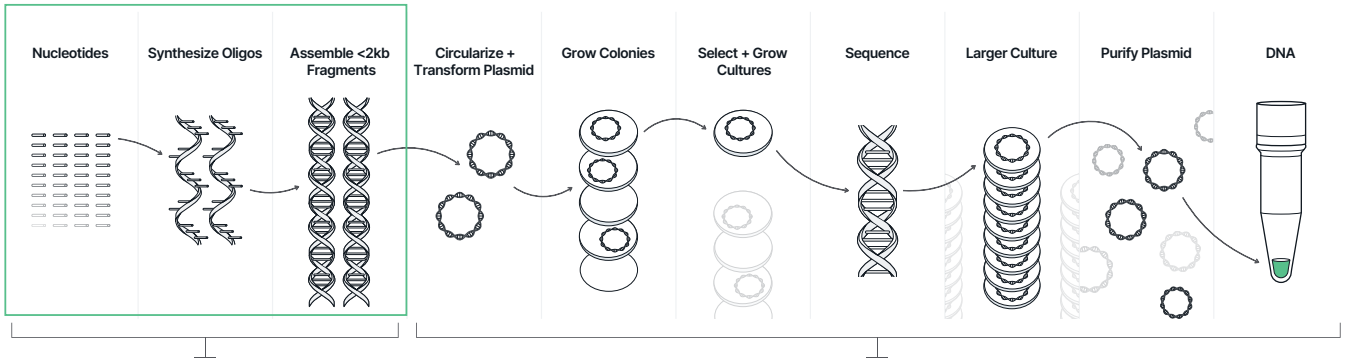
2020s

Breakthroughs in the assembly of DNA lengths >10 kb, error-corrected gene assembly, and cell-free cloning

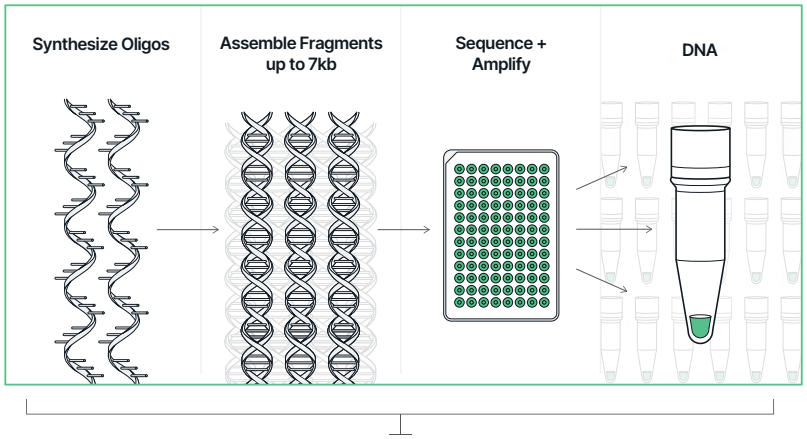
- **2021** FDA approves the first mRNA-based COVID-19 vaccine (Comirnaty, Pfizer-BioNTech))
- **2022** FDA approves the first T-cell receptor (TCR) therapeutic for metastatic uveal melanoma (tebentafusp, Immunocore)
- **2022** FDA approves the first AAV vector-based gene therapy for adults with hemophilia B (Hemgenix, CSL Behring)
- **2022** mRNA therapeutics for genetic diseases including cystic fibrosis enter clinical trials
- **2023** FDA approves first gene therapy for adults with severe hemophilia A (Roctavian, BioMarin Pharma)
- **2023** FDA approves the first CRISPR therapy for sickle cell disease (Casgevy, Vertex Pharma)
- **2023** Elegen launches long, high complexity, clonal-quality ENFINIA™ DNA, produced entirely cell-free

03
The DNA Supply Bottleneck

The lengthy, time-consuming process of assembling and cloning short fragments of DNA is costly, error-prone, and difficult to automate.[5] The approach can take several weeks and even months to perform multiple rounds of construction and cloning required for multi-kilobase constructs.



Conventional gene assembly: 80% of the time required to build synthetic DNA constructs is spent cloning in cells.



Next generation gene synthesis suppliers deliver clonal quality DNA ready for use in as fast as one week.

04
Next-Generation Gene Synthesis is Cell-Free

Elegen, an innovator of DNA manufacturing technology, uses a patented cell-free cloning technology to synthesize double-stranded DNA longer, faster, and with higher complexity than conventional gene synthesis providers offer.

Manufactured in the U.S. and delivered NGS-verified in as fast as 6 business days, Elegen's ENFINIA DNA eliminates the time and resources spent cloning DNA from conventional suppliers. With a reliable and predictable supply of DNA and more time to focus on discovery, researchers iterate designs, gain insights, and advance projects faster than ever before.

> *"We are extremely impressed with Elegen's ability to produce DNA quickly, including difficult sequences."*
>
> **– Alec Nielsen**
> *Co-founder and CEO of Asimov*

TEXAS
The University of Texas at Austin

Researchers like those in the Keatinge-Clay lab use synthetic DNA to engineer and heterologously express polyketide synthases (PKSs) for the *in vivo* biosynthesis of designer polyketides. The sequences used by the lab are long, repetitive, and GC-rich, making them both difficult to amplify from genomic DNA and challenging for conventional gene synthesis providers to produce.

Elegen, using its proprietary cell-free cloning technology, produced a 14,713 bp construct containing a PKS cluster within 9 business days. Having observed successful biosynthesis, the Keatinge-Clay lab saved two weeks of assembly time and labor over their traditional approaches.

05
Advancing AI

Access to high-quality synthetic DNA enables precise, efficient, and scalable experimentation to advance generative models for therapeutic and vaccine development. Here's why it's crucial:

- **Training Data for Generative Models:** Generative models rely on vast, high-quality datasets for accurate learning and prediction. High-quality synthetic DNA can be used to generate a wide array of genetic sequences that mimic real-world diversity, providing models with enriched datasets that improve training accuracy and predictive capabilities.

- **Testing Hypotheses:** Generative models are used to predict the effects of genetic variations and to design novel sequences for therapeutic purposes. With high-quality synthetic DNA, researchers can test predicted sequences in laboratory settings, closing the loop between model predictions and real-world results. This feedback improves the model's accuracy and utility over time.

- **Accelerated Design Cycles:** Precise synthetic DNA allows for rapid prototyping of therapeutic sequences, enabling researchers to test different versions of potential treatments in parallel. This parallel testing speeds up the design cycle, allowing generative models to incorporate experimental outcomes and make optimized suggestions faster than traditional design processes.

- **Reduction in Experimental Noise:** Low-quality or inaccurate synthetic DNA introduces variability, leading to inconsistent results and complicating the generative model's learning process. High-quality DNA minimizes errors, leading to cleaner, more reliable datasets that improve the accuracy and reliability of model predictions.

- **Scalability for Iterative Design:** Synthetic DNA of high quality can be produced at scale, supporting the demands of generative models that require iterative testing. This scalability is crucial in the context of vaccines and therapeutics, where speed is essential, especially in response to emerging pathogens.

- **Exploration of Genetic Space:** High-quality synthetic DNA enables researchers to explore diverse genetic designs and functional areas previously inaccessible. Generative models can suggest novel DNA sequences, which are then synthesized and tested, allowing researchers to explore entirely new therapeutic pathways and mechanisms.

In essence, high-quality synthetic DNA acts as both the fuel and feedback mechanism for generative models, helping unlock more accurate predictions and optimized design of therapeutics and vaccines with unprecedented speed and precision.

Active Learning-Assisted Directed Evolution (ALDE)

Directed Evolution (DE) is a method developed by Nobel laureate Frances Arnold to improve protein fitness for specific applications. However, DE can be less efficient when mutations show epistatic behavior, meaning the effect of one mutation depends on others. A newer approach, Active Learning-assisted Directed Evolution (ALDE), incorporates machine learning into the DE process to address this issue.

In a GEN webinar, Ravi Goel Lai from the Arnold Lab at Caltech discusses how next-gen gene synthesis supports applications of ALDE.

View the Webinar

06
Build versus Buy

Scientists make several decisions and tough compromises when considering how and where to get DNA constructs built. In general, the type of constructs, project budget, and planned timeline will inform these decisions, as well as the lab's infrastructure and experience. The results of these decisions will directly impact the timing of your workflow and the quality of your test cycle.

Think of your last DNA purchase. What choices did you make? Are you spending too much time building DNA templates in-house? Or are you waiting and paying for conventional DNA suppliers to deliver the perfect clone? Once they do, can you go straight into an experiment? As you run through this checklist of common questions and considerations, what could you do to save time, resources, and costs to progress your project?

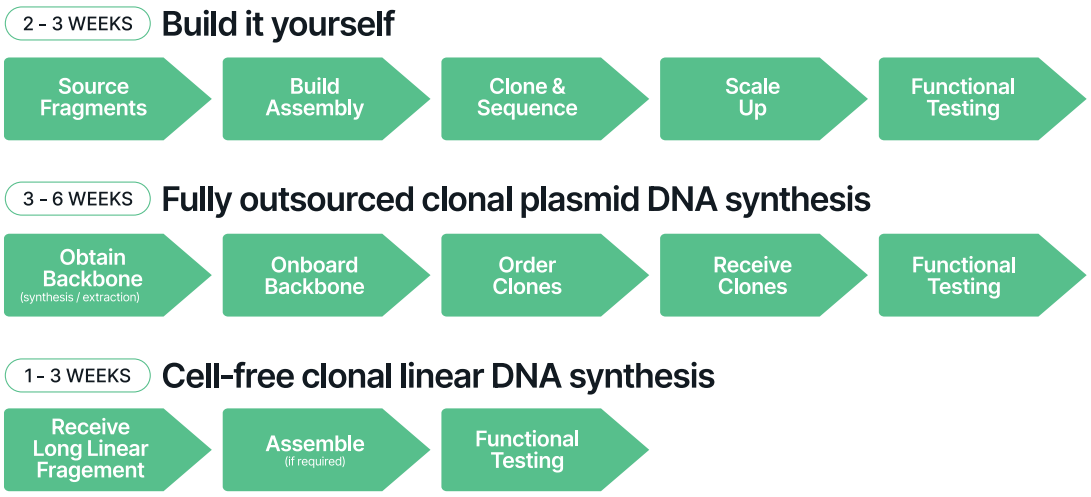
With a better understanding of your application, project constraints, and DNA requirements, you can evaluate multiple DNA supply approaches to assess the impacts of each build vs. buy decision. An internal build approach may deliver your construct quickly but will likely require a significant investment in resources and equipment. Outsourcing DNA builds to a gene synthesis supplier may take more time or add cost depending on the technologies they employ and their manufacturing capabilities.

What DNA Build Strategy Should Account for

Application
- Screening
- Design Optimization
- Functional Testing
- Preclinical Testing
- Clinical Testing

Project Constraints
- Budget
- Deadlines
- Internal Capabilities
- Regulatory Compliance

DNA Requirements
- Grade (e.g. research, GMP)
- Yield
- Purity
- Accuracy
- Length
- Complexity
- Format (e.g. linear, plasmid)
- Quantity of Sequences

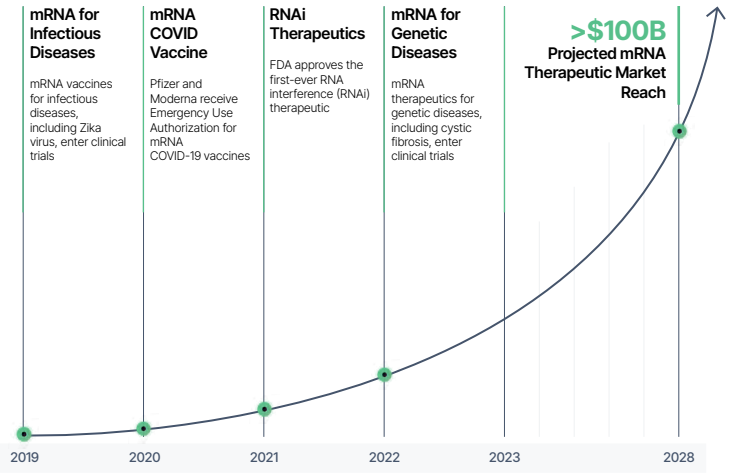


07
The Impacts of Build versus Buy Decisions on R&D

EXAMPLE: TEMPLATE DNA FOR IVT-MEDIATED *m*RNA SYNTHESIS

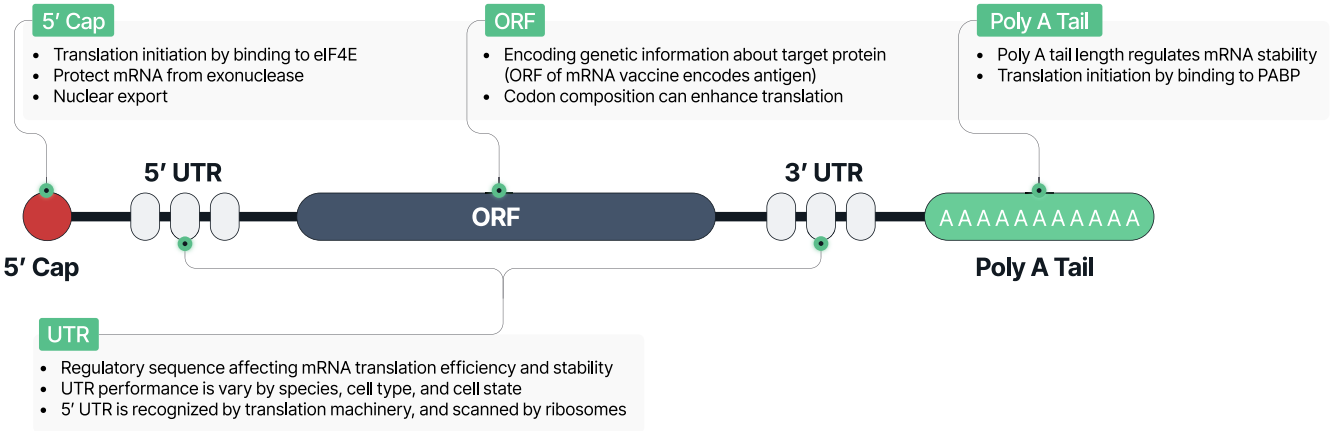
mRNA vaccines and therapeutics show tangible promise as both safe and effective treatments that can be used to solve future public health crises and prevent infectious diseases. Their success hinges the design and production of a single-stranded synthetic RNA molecule through *in vitro* transcription (IVT).[6]

When developing mRNA vaccine or therapy candidates, researchers must often face several tradeoff decisions regarding the supply of template DNA for IVT to sufficiently explore a wide range of designs with a limited budget and tight deadlines.



IVT-Ready DNA for mRNA

mRNA design is driven by iteration of linear DNA that serves as a template for IVT



The image above represents the basic structure of a linear DNA template used for IVT. Note that IVT requires the addition of a poly-A tail of 30 to 160 bases to the 3′ end.

BUILD VS. BUY STRATEGIES

The conventional approach to mRNA production employs IVT of a DNA template that includes a 3′ poly-A tail of 30 to 120 bases. Construction of this DNA template can follow one of several workflows:

1. Cloning a vector containing a poly-A tail

Parts or all of the mRNA encoding portion of the template is inserted into a cloning vector that already includes a poly-A tail.

Tradeoff: *This approach is often considered fast and affordable but is prone to poly-A recombination (i.e., polydispersity) during cloning in bacterial cells, resulting in a heterogeneous population of mRNA molecules and a lower yield of the desired mRNA sequence.*

2. PCR addition of a poly-A tail to cloned DNA

The mRNA encoding portion of the template is cloned in bacterial cells before the addition of a poly-A tail by PCR.

Tradeoff: *This approach adds little time to cloning a vector including a poly-A tail, and avoids the risk of poly-A recombination during cloning. However, the risk of incomplete plasmid linearization or insufficient purification before PCR can result in a lower yield of the desired mRNA sequence.*

3. PCR addition or ligation of a poly-A tail to a linear DNA fragment

The mRNA encoding portion of the template is amplified by PCR prior to the addition of a poly-A tail by PCR.

Tradeoff: *This approach is as fast as any other approach, but some gene synthesis suppliers cannot easily produce linear sequences greater than 2-3 kb without cell-based cloning. For those that can, amplifying the linear template carries the risk of producing side products that result in a lower yield of the desired mRNA sequence.*

With cell-free end-to-end synthesis and amplification, next-gen gene synthesis suppliers provide IVT-ready template DNA up to 7 kb in length and less side product for consistently high mRNA yield and purity.

IVT-Ready Linear DNA Templates – An Easy Decision

Eliminating the variability, cost, and operational complexity associated with cell-based cloning offers developers of mRNA vaccines and therapeutics a distinct advantage over cell-based approaches. The speed, flexibility, and scalability of an end-to-end cell-free mRNA manufacturing approach offers a level of predictability unmatched by conventional methods.

Learn how Elegen produced high-fidelity IVT template in 2 weeks, without cell-based cloning.

View the Poster

EXAMPLE: AAV DELIVERY SYSTEM FOR GENETIC MEDICINES

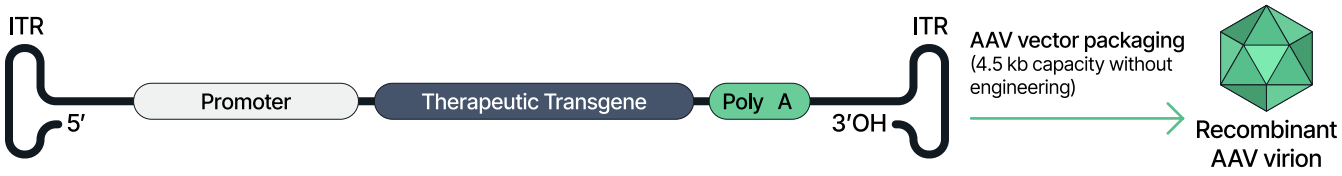
AAV has proven itself as a potent vector for *in vivo* gene transfer into cells and is used for various therapeutic purposes, including gene therapies for cancer and other malignancies, vaccine development, and delivery of CRISPR-Cas9 machinery.

AAV2 is the most commonly used serotype within the AAV family, especially for optogenetics experiments, because of its ability to preferentially infect specific cell types and remain episomal.[8]

The advantages of using AAV for therapy design include:

- Injectability for *in vivo* applications
- Low immunogenicity
- Restricted generation of neutralizing antibodies
- Replication defectiveness

AAV Genome Structure



The basic structure of the AAV viral transfer plasmid consists of two inverted terminal repeats, a promoter that drives the expression of the transgene, and a poly-A tail. The transfer plasmid is accompanied by a Rep/Cap plasmid and a helper plasmid to construct the viral particle.

Gene synthesis is primarily leveraged in the development of plasmids used to transfect the AAV genome into HEK293 cells for the production of viral particles. The AAV genome consists of two open reading frames known as Rep and Cap, which together are flanked by inverted terminal repeats up to 145 bp long.

To produce infectious particles, HEK293 cells are transfected with three separate plasmids: 1) Transfer plasmid containing a promoter and therapeutic transgene flanked by two inverted terminal repeats (ITRs), 2) Rep/Cap plasmid, 3) Helper plasmid. The Rep/Cap plasmid contains genes that are translated into multiple Rep and capsid proteins, which mediate AAV replication and genome packaging to form functional viral capsids.

Since the designs of the Rep/Cap and Helper plasmids are typically unchanged from experiment to experiment, plasmids can be produced at large scale and used across many experiments testing various transgene designs.

The design of the transfer plasmid, however, is subject to frequent iterative change in the course of AAV therapy development. Construction of the transfer plasmid typically involves the insertion of a transgene sequence into a serotype-specific AAV vector containing ITRs and a promoter.

BUILD VS. BUY DECISIONS

Construction of AAV transfer plasmids can follow any one of the following approaches:

1. Internal assembly and cloning
Conserved parts of the assembly are sourced as fragments, stored onsite, and combined with variable parts that are sourced as fragments in realtime. Assembled plasmids are then cloned and sequenced to isolate a sequence-perfect clone before shipment.

Tradeoff: *This approach is often considered fast and affordable but requires a significant investment in equipment and personnel to manage parts supplied by multiple vendors and a complex cloning process.*

2. External plasmid synthesis
Vector backbones containing conserved parts are synthesized and onboarded with an external provider who will synthesize and insert variable parts during cloning to produce sequence-perfect plasmids.

Tradeoff: *Outsourced synthesis of complex plasmid designs can be expensive and time consuming. Weeks to onboard a custom vector is often followed by 3-6 weeks of synthesis and cloning depending on complexity.*

Next-gen gene synthesis providers help boost the throughput and success rates of internal synthesis by providing longer and more complex inserts in as fast as 6 business days. Some will even deliver these inserts as a plasmid in less than 4 weeks.

ENFINIA™ Plasmid DNA

Shipped in just 2-4 weeks, ENFINIA Plasmid DNA incorporates NGS-verified linear DNA inserts produced using Elegen's patented cell-free cloning technology into standardized vectors that are cloned and sequenced before shipment.

Choose from a library of vector backbones and prep scales to suit your project, including options for AAV2 vectors with ITRs and CMV, CAG, or hsyn promoters. Plasmids can be up to 18kb, with certain vector backbones accepting inserts up to 15kb. Built from ENFINIA Linear DNA, these inserts can vary in complexity to explore a wide range of sequences.

Learn how ENFINIA Plasmid DNA can save weeks over conventional clonal gene synthesis.

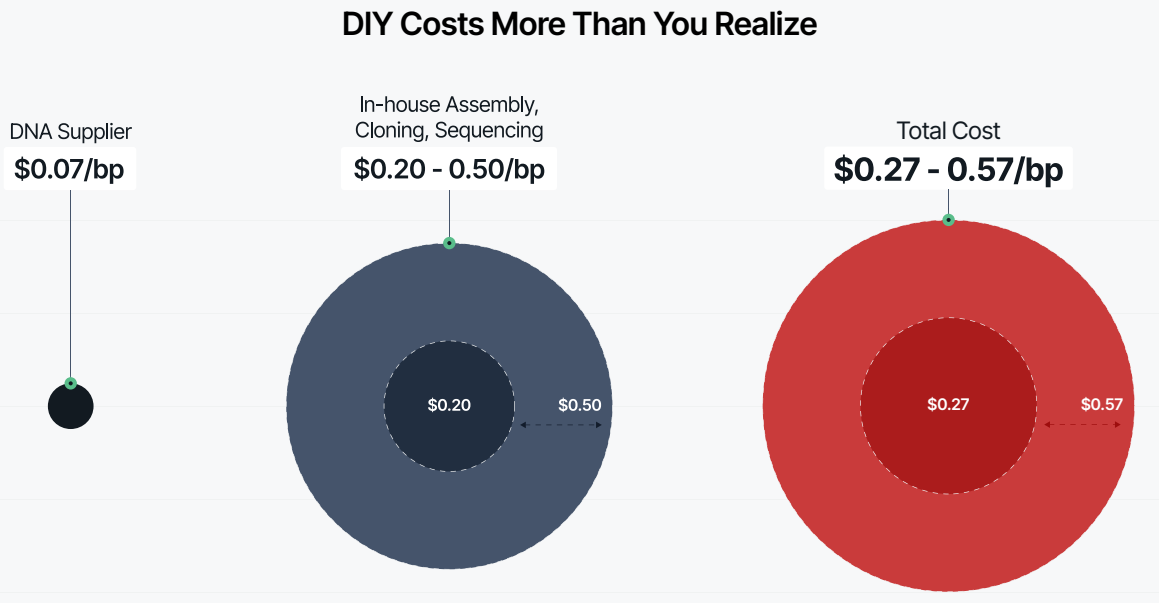
Learn More

08
Evaluating Vendors

Once you've determined the importance of each production factor, final DNA format, and requirements, and built strategy for your application, it's time to research vendors to find the best fit. Your DNA supplier review should go hand-in-hand with your DNA build evaluation. You need to ensure the vendor you select is capable of providing the long-term critical foundation you need to support your current and future research as well as clinical production requirements.

DNA Buyer Pro Tip

Next-generation gene synthesis suppliers that can produce longer, more complex linear DNA parts for plasmid assembly can enable developers to rapidly test a broader range of designs. Instead of piecing together a transfer plasmid from 6 to 8 individual parts, researchers can purchase 1 or 2 parts comprised of multiple elements. With fewer, longer parts, the success rate of assembly increases as well.



WHAT DNA BUYERS NEED TO KNOW

Here, we compare various DNA synthesis providers, focusing on key performance metrics to help you evaluate their potential fit for next project. By examining error rate per bp, turnaround time, synthesis cost, and other key factors, you can decide on the best provider for your needs.

Company	Product	Format	Complexity	Error Rate Per bp	NGS Verified Quality	Turnaround Time for 1 Round of Synthesis	Max Length (kb)	Synthesis Cost	Recommended number of clones to screen
Next Generation Cell-Free Gene Synthesis and Cloning									
Elegen	ENFINA Linear DNA	Long, linear dsDNA	Standard	1:70,000	Yes	6 - 8 days	5.5 kb	$$	0-2
Elegen	ENFINA Linear DNA	Long, linear dsDNA	High	1:70,000	Yes	10 - 15 days	7 kb	$$$	0-2
Chemical/Enzymatic DNA Synthesis and Cell-Based Cloning									
IDT	gBlock	Linear dsDNA fragments	Standard	1:5,000	No	10 - 15 days	3 kb	$$	8-12
IDT	eBlock	Linear dsDNA fragments	Standard	1:5,000	No	1 - 3 days	1.5 kb	$	4-6
IDT	Standard Gene Synthesis	Clonal gene synthesis	Standard	N/A	Yes	8 - 12 days	5 kb	$$$	N/A
ThermoFisher Scientific	DNA Strings	Linear dsDNA fragments	Standard	1:5,000	No	3 - 6 days	3 kb	$$	4-6
ThermoFisher Scientific	Standard Cloned Genes	Clonal gene synthesis	Standard	N/A	Yes	5 - 12+ days	7 kb	$$	N/A
ThermoFisher Scientific	Standard Cloned Genes	Clonal gene synthesis	High	N/A	Yes	5 - 19+ days	12kb	$$$	N/A
Twist BioScience	Gene Fragments	Linear dsDNA fragments	Standard	1:7,500	No	2 - 4 days	5 kb	$	8-10
Twist BioScience	Standard Clonal Genes	Clonal gene synthesis	Standard	N/A	Yes	10 - 15 days	5 kb	$$	N/A
GenScript	Gene Titan	Linear dsDNA fragments	Standard	1:5,000	No	>4 days	1.8 kb	$	8-10
GenScript	Basic Gene Synthesis	Clonal gene synthesis	High	N/A	Yes	> 9 days	200 kb	$$$	N/A
GENEWIZ by Azenta	FragmentGENE	Linear dsDNA fragments	Standard	N/A	No	2 -8 days	3 kb	$$	8-12
GENEWIZ by Azenta	PriorityGENE	Clonal gene synthesis	High	N/A	Yes	8 - 40 days	10 kb	$$$	N/A
ANSA Biotechnologies	ANSADNA Fragments	Linear dsDNA fragments	High	<1:5000	No	>5 days	0.6 kb	$$	4-6
ANSA Biotechnologies	ANSAClonal DNA	Clonal gene synthesis	High	N/A	Yes	>15 days	0.6 kb	$$$	N/A
Synbio Technologies	Fragment XP	Linear dsDNA fragments	Standard	1:5000	No	3 - 4 days	6 kb	$$	8-10
Synbio Technologies	Gene Synthesis	Clonal gene synthesis	High	N/A	Yes	5 - 25 days	6 kb	$$$	N/A

ELEGEN

Unlock Speed, Versatility, and Reliability

Don't waste time with unreliable services, laborious workflows, expensive equipment, and delayed projects.

ENFINIA™ Linear DNA

Cell-Free Gene Synthesis

Linear DNA up to 7 kb shipped in **6 to 8 business days**

NGS-verified and **20x more accurate** than conventional fragments

High complexity synthesis of ITRs, LTRs, GC-rich regions, repeats

"Our cell-free DNA manufacturing technology transforms 'DNA Write' the same way NGS transformed 'DNA Read.' It facilitates and speeds the acquisition of critical experimental inputs."

– **Matthew Hill, PhD**
Founder and CEO, Elegen
Source: Genetic Engineering News (12)

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Sources: 1. Nightingale, Kath. DNA: A timeline of discoveries. BBC Science Focus (2020). https://www.sciencefocus.com/the-human-body/dna-a-timeline-of-discoveries **2.** Hoose, A., Vellacott, R., Storch, M. et al. DNA synthesis technologies to close the gene writing gap. Nat Rev Chem 7, 144–161 (2023). https://doi.org/10.1038/s41570-022-00456-9 **3.** ASGCT. Twenty years of ASGCT and gene therapy. https://www.asgct.org/getattachment/About/History/Timeline-Slides-pdf-version.pdf.aspx?lang=en-US **4.** Hoose A, Vellacott R, Storch M, Freemont PS, Ryadnov MG. DNA synthesis technologies to close the gene writing gap. Nat Rev Chem 7, 144-161 (2023). doi:10.1038/s41570-022-00456-9 **5.** Kosuri, S., Church, G. Large-scale de novo DNA synthesis: technologies and applications. Nat Methods 11, 499–507 (2014). https://doi.org/10.1038/nmeth.2918 **6.** Chaudhary, N., Weissman, D. & Whitehead, K.A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20, 817–838 (2021). https://doi.org/10.1038/s41573-021-00283-5 **7.** Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019 May;18(5):358-378. doi: 10.1038/s41573-019-0012-9. **8.** Labant, MaryAnn. Making AAVs the Go-To for Making Gene Therapies Go. Genetic Engineering & Biotechnology News. 2023 Oct 10. https://www.genengnews.com/topics/drug-discovery/making-aavs-the-go-to-for-making-gene-therapies-go/ **9.** Xu, X., Chen, W., Zhu, W. et al. Adeno-associated virus (AAV)-based gene therapy for glioblastoma. Cancer Cell Int 21, 76 (2021). https://doi.org/10.1186/s12935-021-01776-4 **10.** Syyam, A., Nawaz, A., Ijaz, A., Sajjad, U., Fazil, A., Irfan, S., ... Afzal, S. (2022). Adenovirus Vector System: Construction, History and Therapeutic Applications. BioTechniques, 73(6), 297–305. https://doi.org/10.2144/btn-2022-0051 **11.** Srivastava, A., Mallela, KMG., Deorkar, N., et al. Manufacturing Challenges and Rational Formulation Development for AAV Viral Vectors. Journal of Pharmaceutical Sciences, 110(7), 2609-2624 (2021). https://doi.org/10.1016/j.xphs.2021.03.024 **12.** Labant, MaryAnn. Enzymatic DNA Synthesis: Shorter Waits, Longer Strands. Genetic Engineering & Biotechnology News. 2024 July 1. https://www.genengnews.com/topics/omics/enzymatic-dna-synthesis-shorter-waits-longer-strands/

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